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Review

Recognition of immunoglobulins by Fcγ receptors

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Abstract

Fc receptors mediate antibody dependent inflammatory response and cytotoxicity as well as certain autoimmune dysfunctions. Fcγ receptors interact with IgG antibodies by binding the Fc portion of the antibody in asymmetric fashion creating a 1:1 receptor-ligand stoichiometry. Regions of the C-terminal domain of Fc receptors including the BC, C′E, FG loops, and the C′ β-strand interact with immunoglobulins. The lower hinge region of the antibody contributes most of the binding to the low affinity Fcγ receptors. Carbohydrates attached to the conserved glycosylation site on Fc portion of an antibody are critical to the recognition of immunoglobulins by the low affinity Fcγ receptor. They are likely to function as a substitution for the hydrophobic core to preserve an optimal lower hinge conformation for the receptor binding. Subtype specificities of FcγRIII receptor probably are determined by the length of the lower hinge regions of immunoglobulins, but not their amino acid composition as revealed by the binding study of the lower hinge peptides. These studies also paved a new way for designing of novel therapeutic compounds in fighting autoimmune diseases. Published by Elsevier Science Ltd.

Keywords: Fc receptors; Immunoglobulins; Immune complexes; Crystal structures; FcγRIII

1. Introduction

Immunoglobulin Fc receptors (FcR) are expressed on all hematopoietic cells and play a crucial role in immune defense by providing a link between antibody–antigen complexes and cellular effector machinery. Binding of immune-complexes to FcR activates effector cells leading to phagocytosis, endocytosis of IgG-opsonized particles, the release of inflammatory mediators, and antibody dependent cellular cytotoxicity (ADCC) (Young et al., 1984; Anderson et al., 1990; Titus et al., 1987; Chan and Sinclair, 1971; Phillips and Parker, 1983). FcR have been described for all classes of immunoglobulins: FcγR and neonatal FcR (FcRn) for IgG, FcεR for IgE, FcαR for IgA, FcδR for IgD, and FcμR for IgM (Unkeless et al., 1988; Fridman, 1991; Hulett and Hogarth, 1994; Daeron, 1997; Ravetch and Kinet, 1991). Of these receptors leucocyte FcγR and FcεR were characterized most extensively. Structurally, all known FcR belong to immunoglobulin superfamily, except for the FcRn and FcεRII, which are structurally related to class I major histocompatibility antigens and C-type lectins, respectively. Among them, FcγRI and FcεRI are high affinity FcR with dissociation constants ranging from 10^{-8} to 10^{-10} M (Ravetch and Kinet, 1991; Daeron, 1997; Hulett and Hogarth, 1994). All other IgG receptors, such as FcγRII and FcγRIII, are low affinity receptors with dissociation constants 10^{-5}–10^{-7} M. The high affinity receptors FcεRI and FcγRI are activated by monomeric immunoglobulins. Under physiological conditions the low affinity receptors FcγRII and FcγRIII require multivalent immune complexes for their activation. In addition to the affinity variations among the receptors, each Fcγ receptor displays distinct IgG subtype specificities. For example, FcγRIII binds IgG1 and IgG3 better than IgG2 and IgG4 (Tamm and Schmidt, 1997). This review focuses on the structure and function of Fcγ receptors.

2. Features and functions of Fcγ receptors

Three subclasses of receptors, denoted as A, B, and C, are known for FcγRI and FcγRII, and two subclasses A and B are described for FcγRIII (Daeron, 1997). All Fcγ receptors are type I transmembrane glycoproteins with a ligand-binding α subunit consisting of two (FcγRIIA, B, C and FcγRIIIA, B) or three (FcγRIA, B, C) C2-type immunoglobulin-like domains. All Fcγ receptors show

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high degree of sequence identity in their extracellular portion (50–96%), but differ significantly in their cytoplasmic domains (Ravetch and Kinet, 1991). Some Fcγ receptors contain the immunoreceptor tyrosine-based activation motif (ITAM) (FcγRIIA, C) or the corresponding inhibitory motif (ITIM) (FcγRIIB) in their cytoplasmic tails. Others (FcγRI and FcγRIIIA) require the association of the ITAM containing FcR common γ chain or T cell receptor ζ chain for signaling. Activating and inhibitory Fcγ receptors are co-expressed on macrophages, monocytes, neutrophils and eosonophils (Ravetch and Bolland, 2001). Different from other Fcγ receptors, FcγRIIIB is expressed exclusively on neutrophils. It contains no signaling component and is anchored by a glycosyl–phosphatidylinositol linker to the plasma membrane. Presumably, FcγRIIIB acts synergistically with other receptors like FcγRIIA and uses their signaling apparatus for signal transduction and cell activation (Unkeless et al., 1995; Kimberly et al., 1990; Galon et al., 1996). A soluble form of FcγRIIIB was reported to activate the CR3 complement receptor dependent inflammatory process (Galon et al., 1996).

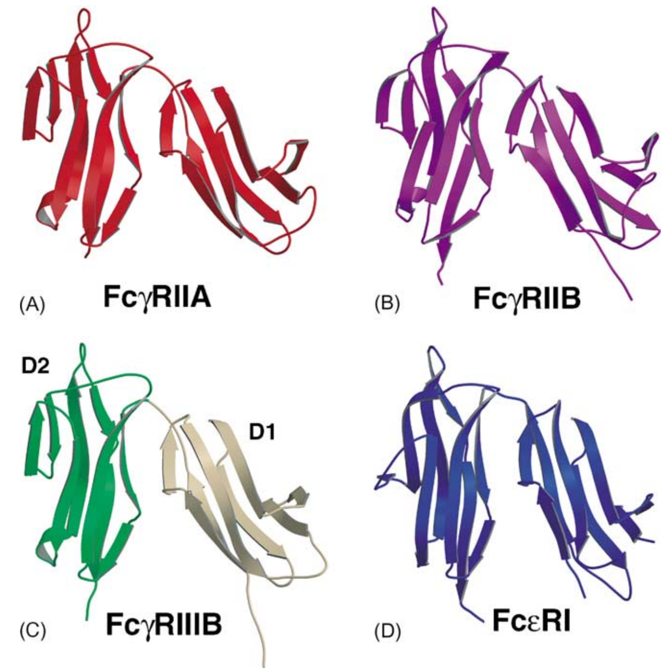

Fig. 1. Structures of ligand-free FcR: (A) FcγRIIA, painted red; (B) FcγRIIB, colored magenta; (C) FcγRIII, domains D1 and D2 are shown in green and beige, respectively; (D) FcεRI, shown in purple. All ribbon figures are prepared using the program MOLSCRIPT (Kraulis, 1991) and Raster3D (Merritt and Bacon, 1997).

3. Crystal structures of Fc receptors

Crystal structures of the extracellular portion of three ligand-free Fcγ receptors: FcγRIIA (Maxwell et al., 1999), FcγRIIB (Sondermann et al., 1999), and FcγRIII (Zhang et al., 2000; Sondermann et al., 2001) as well as the structure of ligand-free FcεRI (Garman et al., 1998) have been solved to date. Their overall structures are remarkably similar to each other and can be superimposed with root mean square (r.m.s.) deviations of 0.9–1.1 Å between different Fcγ receptors and 1.6 Å between FcγRs and FcεRI. They all consist of two Ig-like domains (D1 and D2) with an acute interdomain hinge angle of 55° for FcγRIIA, 52° for FcγRIIB, and 50° for FcγRIII (Fig. 1). Compared to other Ig-like structures, such as the hematopoietic receptors, killer cell immunoglobulin-like receptors (KIR), and adhesion receptors, Fcγ receptors have 15–30° smaller hinge angles and the position of their N-terminal domain D1 with respect to the C-terminal domain D2 is opposite to other receptors (Fig. 2). The interdomain linkers of all FcR are three residues shorter than those of KIR and growth hormone receptors.

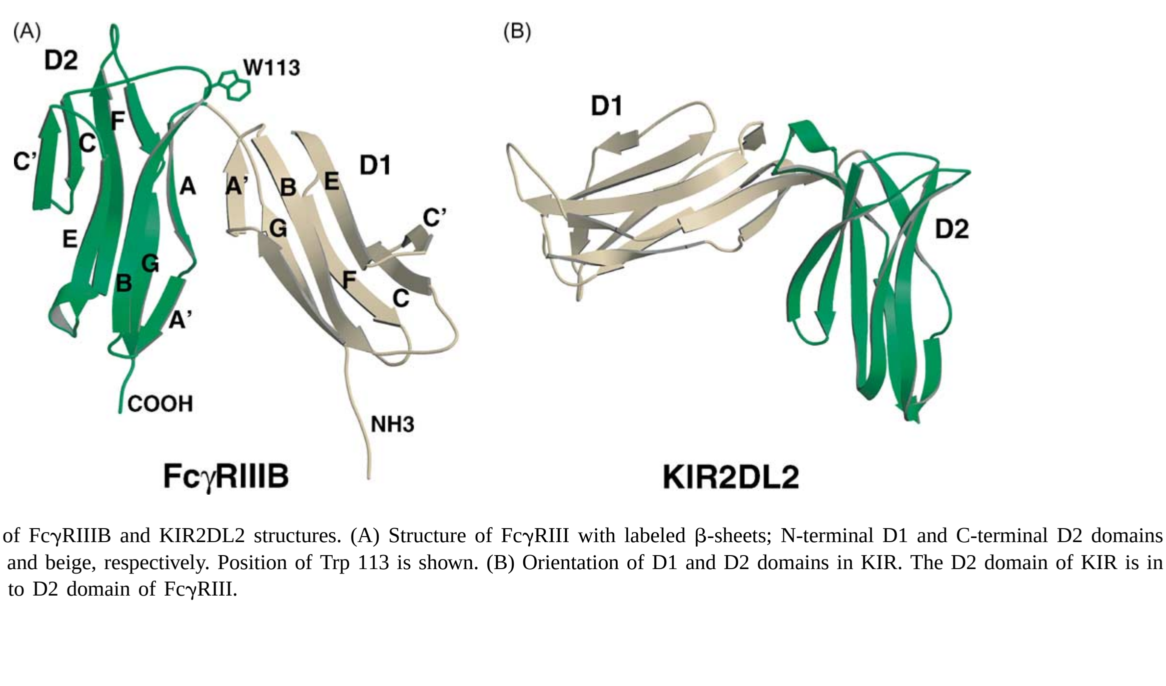

Fig. 2. Comparison of FcγRIIIB and KIR2DL2 structures. (A) Structure of FcγRIII with labeled β-sheets; N-terminal D1 and C-terminal D2 domains are colored in green and beige, respectively. Position of Trp 113 is shown. (B) Orientation of D1 and D2 domains in KIR. The D2 domain of KIR is in a similar orientation to D2 domain of FcγRIII.

This probably prevents FcγRs from adopting the KIR-like D1/D2 domain arrangement. In addition, the BC loop of the D2 domain forms a cap over the D1 domain with a conserved tryptophan residue Trp 113 (Trp 110 in FcγRIIB) from the BC loop wedged into one end of the D1 domain further restricting the interdomain hinge movements (Fig. 2). The interdomain hinge angle is also stabilized by a conserved salt bridge that connects Asp 23 of the D1 domain to His 111 of the D2 domain, and an interdomain hydrophobic core that consists of mostly conserved residues Trp 16, Leu 20, Gln 94, Arg 109, His 111, Ser 112, and Trp 113.

4. The structure of Fc receptor ligands

The structures of Fc fragments complexed with protein A, protein G, rheumatoid factor, neonatal FcR, and a 13 residue peptide as well as an intact mouse antibody IgG2a, have been reported to date (Deisenhofer, 1981; Sauer-Eriksson et al., 1995; Corper et al., 1997; Martin et al., 2001; DeLano et al., 2000; Harris et al., 1992). An Fc fragment contains two identical chains, each with two C1-type immunoglobulin domains C_H2 and C_H3. The overall shape of Fc resembles a horse-shoe with two C_H3 domains packed tightly against each other at the bottom of the horse-shoe and two C_H2 domains forming the opening of the horse-shoe. Ordered carbohydrate moieties are attached to the conserved glycosylation site Asn 297 on both chains of Fc. The intact Fc portion of an antibody shows an intrinsic two-fold symmetry relating the two chains.

5. Structure of the FcγRIII–Fc complex

Recently, the crystal structure of a human FcγRIII in complex with the Fc portion of a human IgG1 has been determined to 3.0 Å (Radaev et al., 2001) and to 3.2 Å resolution (Sondermann et al., 2000) (Fig. 3). The structure was solved in two space groups $P2_12_12_1$ and $P6_522$ with different crystal packings and different solvent contents. However, the receptor to Fc binding stoichiometry in both crystals remains 1:1, consistent with earlier binding studies using non-equilibrium and equilibrium gel filtration experiments (Zhang et al., 2000; Kato et al., 2000). The conformation of the FcγRIII–Fc complex, including the carbohydrate moieties attached to the glycosylation site on Fc, is essentially identical between the crystal forms. The receptor binds to the horse-shoe opening of the Fc making contacts to the lower hinge regions of both chains of the Fc (Hinge-A and -B) (Fig. 3). Such a binding breaks the dyad symmetry of the Fc creating an asymmetric interface whereby the identical residues from Hinge-A and -B interact with different, unrelated surfaces of the receptor. It also excludes the possibility of having a second receptor interacting with the same Fc molecule, resulting in a 1:1 stoichiometry for the receptor-Fc recognition.

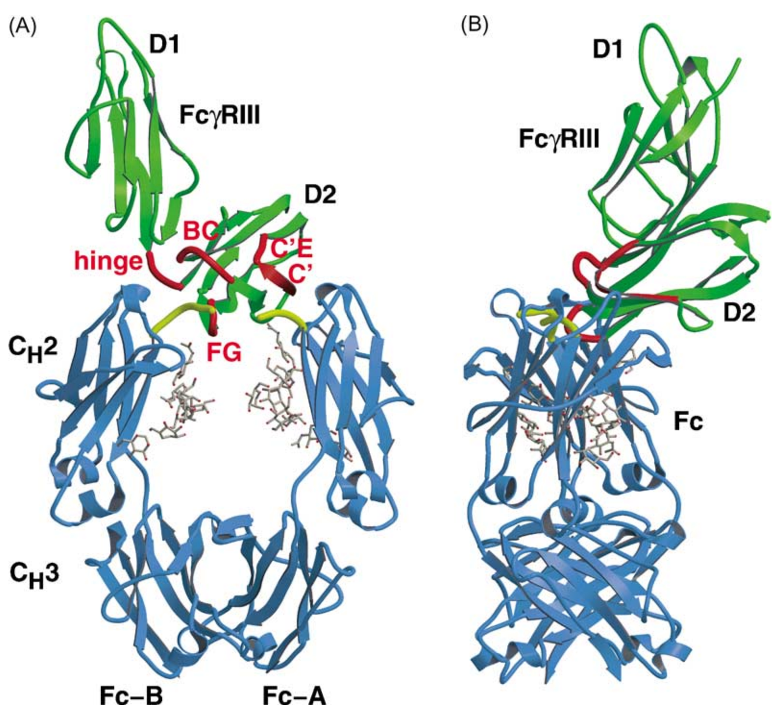

Fig. 3. Structure of the FcγRIII complexed with Fc portion of IgG1: (A) front view of the complex; (B) side view of the complex rotated ≈90° compare to the front view. FcγRIII is shown in green, Fc in cyan. Secondary structure elements of binding region of FcγRIII are colored red. Lower hinge regions on Fc are painted yellow. Positions of C_H2 and C_H3 domains of Fc are marked. Carbohydrate moieties attached to the conserved glycosylation site Asn 297 on Fc are painted grey.

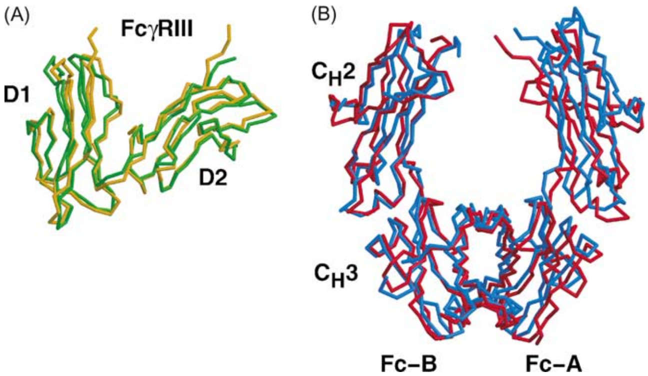

Fig. 4. A superposition of a carbon traces of FcγRIII and Fc in the receptor-Fc complex with ligand-free receptor and Fc. (A) A superposition of FcγRIII from the receptor-Fc complex (green) with ligand-free receptor (orange). (B) A superposition of Fc from the receptor-Fc complex (cyan) with the structure of unbound Fc (red).

No significant change in the overall receptor conformation is observed upon complex formation. The structure of the Fc bound receptor (Radaev et al., 2001) can be readily superimposed onto the ligand-free receptor (Zhang et al., 2000) resulting in r.m.s. differences between the individual domains of 0.6–0.8 Å among all Cα atoms (Fig. 4A). The hinge angle between the N-terminal D1 and the C-terminal D2 domains is 60°, slightly larger than the 50° value observed in the ligand-free receptor. The conformation of the Fc fragment in the complex also does not differ significantly from that of the free Fc (Deisenhofer, 1981) and from that of a murine intact IgG2a antibody (Harris et al., 1992) (Fig. 4B). However, the two-fold symmetry relating the two chains of Fc in unliganded Fc structures is no longer retained in the structure of the complex. The horse-shoe shaped Fc is slightly more open at the N-terminal end of the C_H2 domains in the FcγRIII–Fc structure compared to other known structures of Fc. The hinge angle between C_H2 and C_H3 domains of chain A is about 100°, approximately 10° larger than the corresponding angle in chain B and in the structures of receptor-free Fc.

6. FcγRIII–Fc binding interface

Approximately 1720 Å^2 solvent accessible area is buried upon FcγRIII–Fc complex formation. All contacts to Fc are made exclusively through D2 domain of the receptor whereas its D1 domain is positioned above, but makes no contact with the B chain of Fc (Fig. 3). The interface of the complex consists of the hinge loop between the D1 and D2 domain of the receptor, the BC, C′E and FG loops and the C′ β-strand of the D2 domain. The BC loop is positioned across the horse-shoe opening of Fc making contacts to residues of both Hinge-A and -B. The C′-strand is situated atop chain A of the Fc positioning the C′E loop into contact with the residues of Hinge-A. The receptor FG loop protrudes into the opening between the two chains of Fc (Fig. 3). All three receptor loops (BC, C′E and FG) were implicated in Fc binding through the studies of chimeric FcγRII/FcεRI receptors and through site directed mutagenesis (Hulett et al., 1994, 1995; Cook et al., 1997). On the Fc side of the complex, interactions with the receptor are dominated by residues Leu 234–Pro 238 of the lower hinge, consistent with the results from earlier mutational studies (Tamm and Schmidt, 1997). Together, Hinge-A and -B contribute approximately 60% of the overall receptor-Fc interface area. Interestingly, both

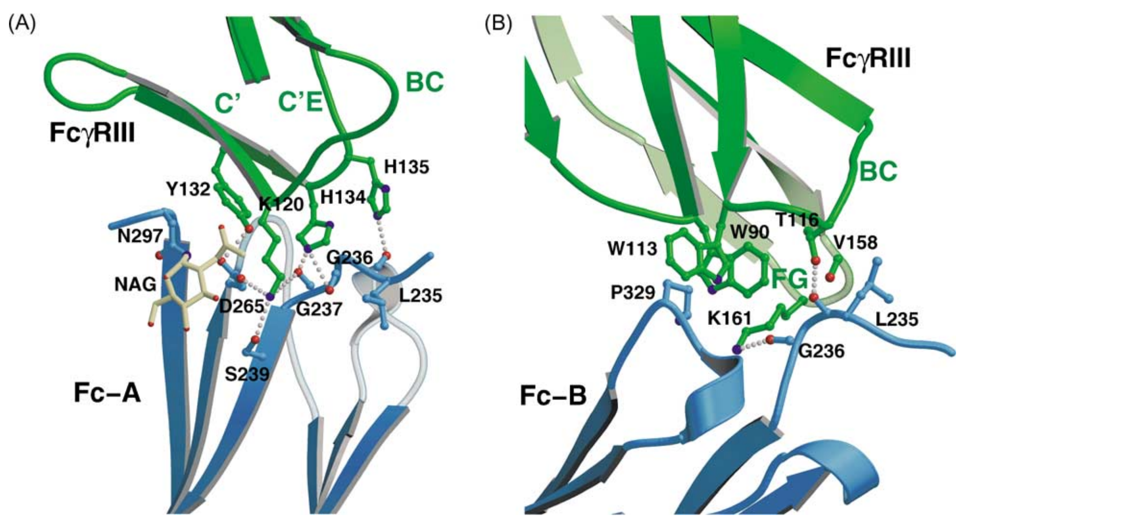

Fig. 5. FcγRIII–Fc interface. (A) Interactions between FcγRIII (green) and chain A of Fc (cyan). The glycosylation residue, Asn A297, and *N*-acetylglucosamine (NAG) that interacts with the receptor are shown. (B) Interactions between FcγRIII (green) and chain B of Fc (cyan). The side chain of Val 158 is omitted for clarity. There is a hydrogen bond between carbonyl group of Val 158 and backbone nitrogen of Gly B236 that is not shown in the picture. Residues are colored by molecule. Important hydrogen bonds are represented by dotted lines. The BC, C′E and FG loops as well as the C and C′ β-strands of FcγRIII which play an important role in the interactions are labeled. Some secondary structure elements of Fc lying behind and not contributing in the binding are shown as semi-transparent. Carbohydrate moieties have been omitted for clarity.

Hinge-A and -B are found disordered in all unbound Fc structures, including the structure of an intact mouse IgG2a (Harris et al., 1992; Deisenhofer, 1981; Sauer-Eriksson et al., 1995; DeLano et al., 2000). In contrast, the same Hinge-A and -B in the current FcγRIII–Fc complex are clearly visible in the electron density maps of both crystal forms suggesting that the binding of FcγRIII stabilizes the lower hinge conformation of Fc.

Overall, key features of the receptor-Fc interface are well preserved among all FcR with possible hinge conformational adjustment for each receptor-Fc pair. The interface between FcγRIII and the A chain of Fc is dominated by hydrogen bonding interactions whereas hydrophobic interactions occur primarily between the FcγRIII and the B chain of Fc. A network of nine hydrogen bonds, including both the main chain and the side chain ones, is formed between the receptor and Fc (Fig. 5). Seven of them are distributed across the receptor and Fc-A interface and two are at the receptor and Fc-B interface. A H134A mutant of FcγRII has been shown to reduce the Fc binding drastically (Hulett et al., 1995),

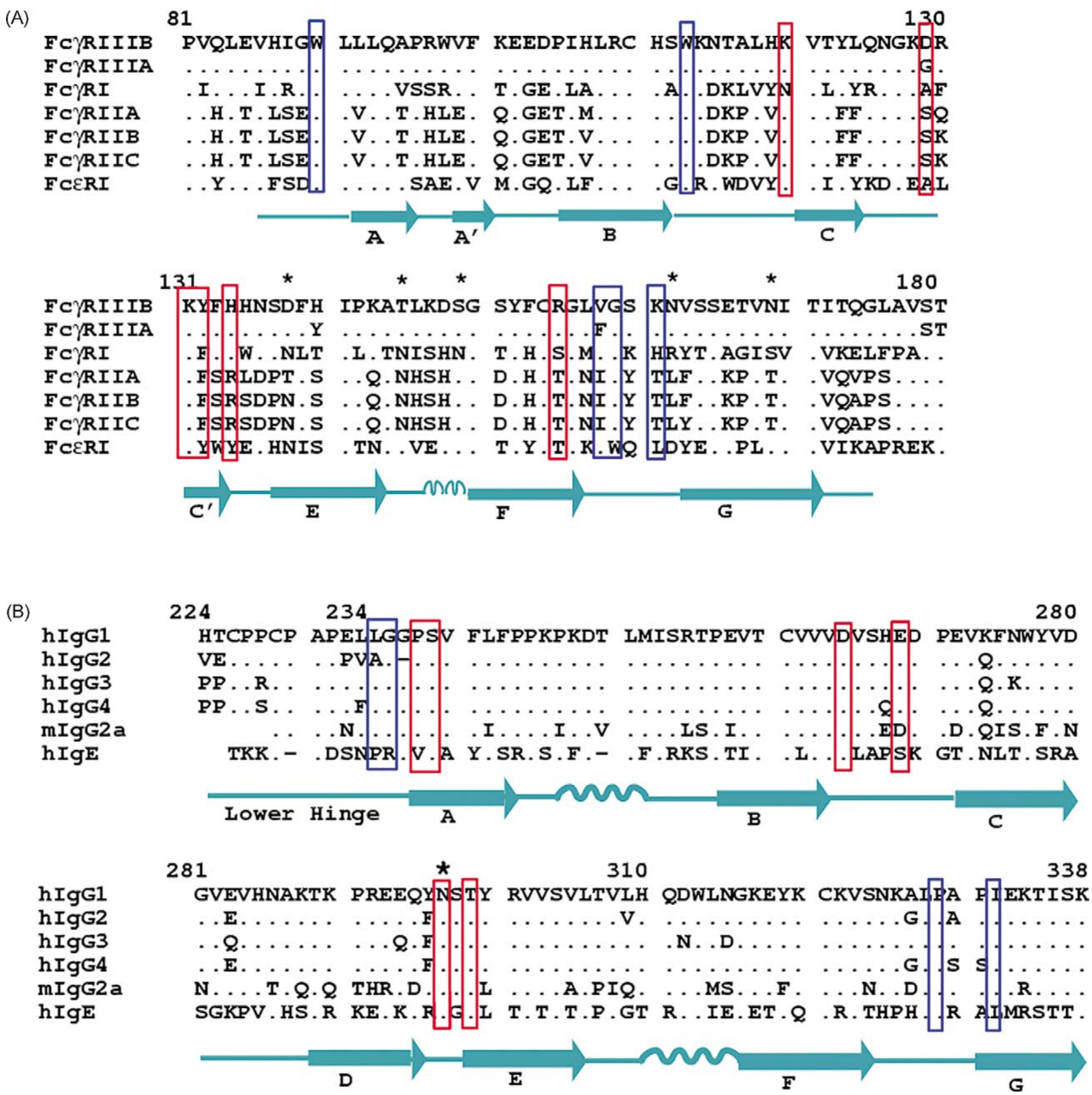

Fig. 6. (A) Sequence alignment of the membrane proximal domain of human FcR. The secondary structure elements (arrow for β-strands and squiggle for α-helices) are indicated under the sequence. Residues identical to the sequence of FcγRIIIB are shown by (...) and gaps in sequence are shown by (–). Residues contacting the A and B chains of the IgG1 Fc are highlighted in red and blue boxes, respectively. The predicted N-linked glycosylation sites are indicated by (*). (B) Sequence alignment of the lower hinge and C_H2 regions of human IgG1, 2, 3, 4, a mouse IgG2a and a human IgE Cε3 region. The residues contacting FcγRIII are highlighted in red and blue boxes for A and B chains of the Fc, respectively.

would result in the loss of two interface hydrogen bonds, illustrating the importance of the interface hydrogen bonding network to the stability of the complex.

A proline sandwich is formed between Trp 90 and Trp 113 of the receptor, both conserved in all human Fcγ and Fcε receptors sequences, and Pro B329, invariant among all Fc sequences (Figs. 5 and 6). This hydrophobic core extends further to include Val 158, the aliphatic portion of Lys 161 from the receptor and Leu B235 from Hinge-B. Mutations of both Trp 113 and Lys 161 in FcγRIII lead to the loss in receptor function (Tamm et al., 1996; Hibbs et al., 1994). The side chain of Leu B235 on the Fc packs tightly against Gly 159 of the receptor leaving little space to accommodate any residues larger than Gly at this position. A Gly to Ala mutation on the chimeric FcγRII resulted in complete disruption of Fc binding highlighting the steric constrain imposed to Gly 159 (Hulett et al., 1994). Of particular interest is Trp 113 of the receptor, to which a Phe substitution resulted in the loss of Fc binding. In addition to be a part of the interface hydrophobic core this tryptophan is also positioned as a wedge inserted into the D1 domain to provide pivotal interactions stabilizing the acute receptor D1/D2 domain hinge angle. A W113F mutation would relieve the pivotal wedge and lead to a disruption in binding by altering the orientation between the D1 and D2 domains.

7. Comparison of the Fc receptor-Fc complex structures

The structural comparison between FcγRIII–Fc complex (Radaev et al., 2001) and FcεRI–Fc complex (Garman et al., 2000) shows a similar mode of receptor-ligand recognition. The two complexes can be superimposed with r.m.s. deviations of 1.5 Å between all the Cα atoms. Most of the structural differences are resulted from the small variation between the C_H2–C_H3 and Cε3–Cε4 inter domain hinge angle. This angle is approximately 10° smaller in the FcεRI–Fc complex structure resulting in a slightly closed conformation of Fc compared to the FcγRIII–Fc complex (Fig. 7A). The interface area buried in the high affinity FcεRI–Fc complex (1850 $Å^2$) is slightly larger than that in the low affinity FcγRIII–Fc complex (1720 $Å^2$). This is primarily due to a fact that the interactions between the receptor and the non-lower hinge residues of Fc are more extensive in the high affinity than in the low affinity complex. Of the total interface area of the Fc, the lower hinge and non-lower hinge regions contribute 870 and 750 $Å^2$, respectively, in the FcγRIII–Fc structure. The carbohydrates attached to the conserved glycosylation site Asn 297 on both chains of Fc contribute about 100 $Å^2$ to the interface. In the FcεRI–Fc structure the corresponding lower hinge and non-lower hinge regions contribute 740 and 1110 $Å^2$, respectively. This shows that the interface area contributed by non-lower hinge residues in the high affinity receptor-ligand complex is significantly more than that in the low affinity complex. Structurally, the lower hinge of IgE–Fc adopts a very different conformation as that of IgG–Fc in their respective receptor complexes (Fig. 7B). This conformation difference may enable the high affinity FcεRI to interact more extensively with its ligand.

Although the overall pattern of the receptor-Fc interactions is preserved between the FcγRIII–Fc and FcεRI–Fc complexes, significant differences are also observed. First, there are more extensive hydrophobic interactions between the FcεRI and IgE–Fc than between FcγRIII and IgG–Fc. While the tryptophan-proline sandwich formed by W90, W113 of FcγRIII and P329 of Fc-B (the corresponding W87, W110 and P426 residues in FcεRI–Fc) is preserved in both structures, additional hydrophobic residues are found in both FcεRI and IgE–Fc to form a more extensive hydrophobic interface contact in the FcεRI–Fc complex compared to FcγRIII–Fc complex. Second, more hydrogen bonds and salt bridges exist at the FcεRI–Fc interface compared to FcγRIII–Fc. Interestingly, the hydrogen bonds in the FcεRI–Fc interface are formed mostly between the side chain atoms whereas those in the FcγRIII–Fc interface are formed primarily between the main chain atoms or between the main chain and side chain atoms. There are two salt bridges K117-D362 (chain A) and E132-R334 (chain A) observed between FcεRI and Fc but only one, K120-D265 (chain A) is found between FcγRIII and Fc. The comparison suggests that multiple interactions contribute to the observed receptor-ligand affinity difference and that the high affinity recognition shows more extensive hydrophobic interface area as well as more prominent electrostatic interactions. In the case of the high affinity IgG receptor-FcγRI, the third extracellular Ig-like domain in the α subunit is believed to contribute to the high affinity of the receptor (Allen and Seed, 1989).

8. IgG subtype specificities

Fcγ receptors display IgG subtype specificities. For example, human FcγRIII binds IgG1 and IgG3 tighter than IgG2 and IgG4 (Ravetch and Kinet, 1991; Tamm and Schmidt, 1997). Previous mutation studies of IgG2 binding to human high affinity receptor-FcγRI concluded that the entire lower hinge sequence was required to restore the IgG1 binding affinity in IgG2,whereas point mutations in IgG1 hinge residues resulted in a loss of the receptor binding (Chappel et al., 1991). Most of the Fc residues of the contact region are conserved among the IgG sequences (Fig. 6B, residues boxed in blue and red), suggesting a conserved binding site for all human IgGs. These binding residues, with the exception of a Glu 269 to Asp replacement, are also conserved in murine IgG2a consistent with it being a ligand for human Fcγ receptors. Lower hinge region of IgG, in particular residues L234–L235–G236–G237–P238, was earlier identified as crucial for binding to FcγR (Tamm and Schmidt, 1997). The sequence differences among the IgG subclasses exist primarily at the lower hinge region.

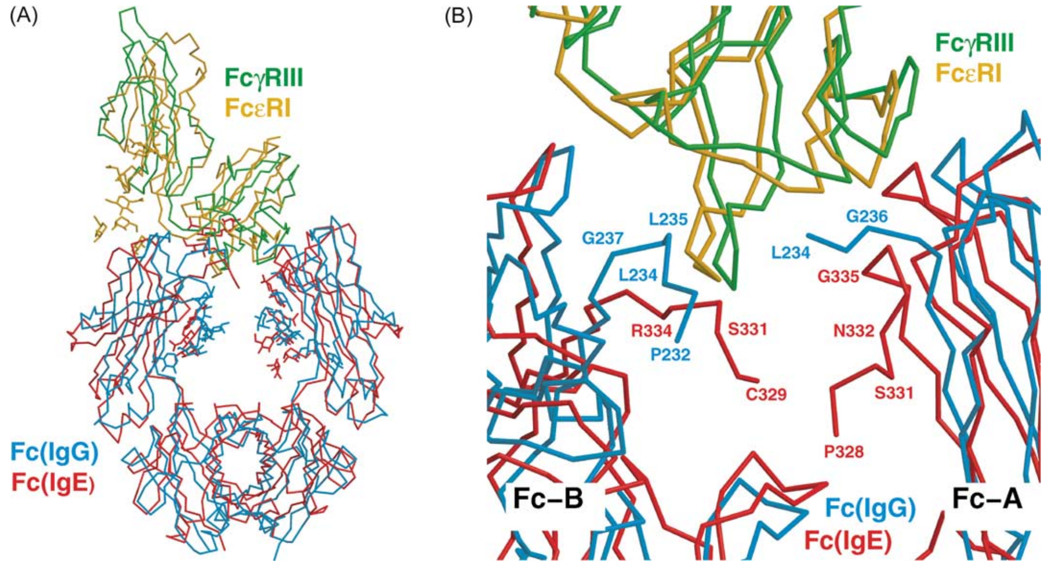

Fig. 7. Superposition of the structures of FcγRIII–Fc and FcεRI–Fc complexes (PDB accession codes (1IIS and 1F6A). (A) Superposition of the structures of FcγRIII–Fc (green and cyan for FcγRIII and Fc, respectively) and FcεRI–Fc (orange and red for FcεRI and Fc, respectively). (B) A close up view of superposition of FcγRIII–Fc (green and cyan) and FcεRI–Fc (red and orange) complexes in the interface area. FcγRIII and IgG–Fc are painted green and cyan, FcεRI and IgE–Fc are colored orange and red, respectively. The lower hinge regions are labeled. The view is identical to panel A.

Human IgG2 has a Val–Ala at positions 234 and 235, instead of Leu–Leu as observed in IgG1 and IgG3, and a one residue deletion at position 237 of the corresponding IgG1. Human IgG4 has a Phe at position 234 (Fig. 6B). In addition, IgG2 and IgG4 sequences contain a three residue deletion relative to IgG1 at the N-terminal end of the lower hinge, possibly restricting the lower hinge conformation.

A study on the binding of peptides with the sequences of lower hinges of IgG1, IgG2, and IgG4 (denoted as pIgG1, pIgG2, and pIgG4) to FcγRIII was carried out in an attempt to explain the receptor binding specificities (Radaev and Sun, 2001). In this case individual amino acid contribution to the receptor affinity is separated from the effect of their environment, namely, the length of lower hinge in an intact antibody. The receptor-peptide binding constants range from 100 to 400 μM (Fig. 8). The results of this study show that pIgG2 and pIgG4 have nearly the same affinity to FcγRIII as pIgG1. Replacing Leu with Phe in pIgG4 or changing Glu–Leu–Leu–Gly to Pro–Val–Ala in pIgG2 in addition to a single residue deletion makes little difference in their affinity to the receptor. This suggests that factors other than the lower hinge amino acid composition play an important role in determining the weaker binding affinity of IgG2 and IgG4 to FcγRIII (compare to IgG1). Earlier, it has been proposed that the overall length of the lower hinge may be important to the receptor IgG subtype specificity (Tamm and Schmidt, 1997), since the hinges of IgG1 and IgG3 are about three residues longer than those of IgG2 and IgG4. It is possible that the shorter lower hinges of IgG2 and IgG4 are constrained in conformation that prohibits optimum receptor binding. Other factors, such as the glycosylation at

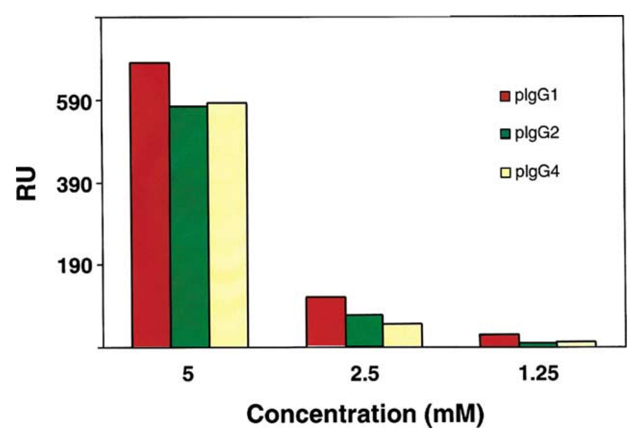

Fig. 8. Binding of the lower hinge peptides to immobilized FcγRIII. The three sets of experiments correspond to 5, 2.5 and 1.25 mM peptide concentrations (from left to right). Amino acid sequences for the peptides are: pIgG1 (CPAPELLGGPSV), pIgG2 (PPVAGPSV), and pIgG4 (PEFLGGPSV). No detectable binding were observed for pALA (sequence AAADAAAAL, was used as a control) at all three concentrations.

Asn 297, which varies among the IgG subtypes, may also contribute to the observed receptor specificity (Jefferis et al., 1998). Residues outside the lower hinge region but in the vicinity of receptor interface can also influence the receptor binding preference (Kato et al., 2000).

9. Peptidyl inhibitors of Fc receptor function

Certain autoimmune diseases, such as rheumatoid arthritis, results from the activation of Fcγ receptors by

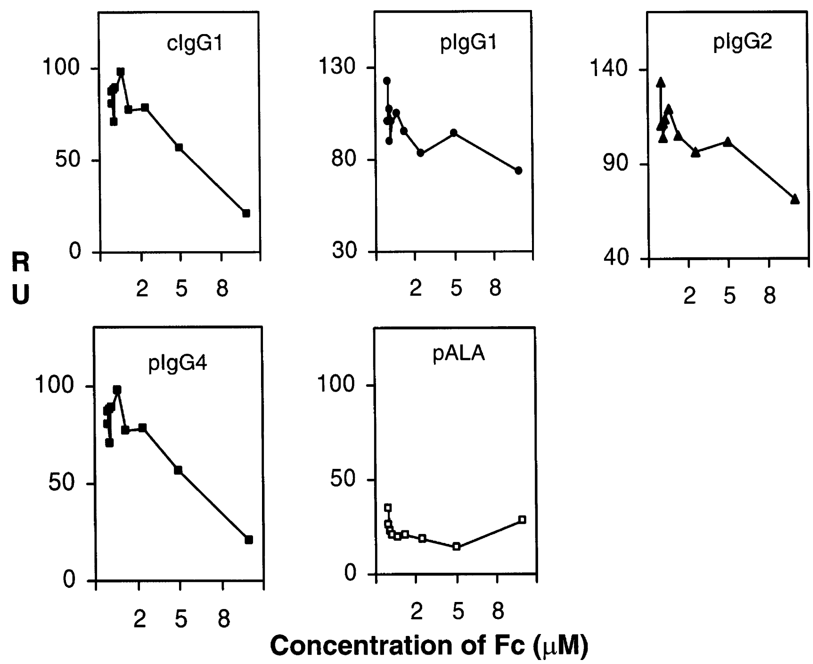

Fig. 9. Effect of peptide competition on FcγRIII binding to Fc. Peptides pIgG1, pIgG2, pIgG4, and cIgG1 (disulfide bonded pIgG1) were immobilized on CM5 sensor chip. pALA was used as a control. Mixtures of 10 μM FcγRIII with various concentrations of Fc from 10 to 0.02 μM were used as analytes. RU indicates binding of FcγRIII to peptide. Dependency of the Fc to FcγRIII binding on the Fc concentration indicates that the lower hinge peptides indeed compete with Fc for the binding to the receptor.

auto-antibodies (Vaughan, 1993). Under these circumstances, the ability to inhibit the receptor activation should help to control the antibody mediated auto-inflammatory response.

Since the lower hinge regions of IgG are dominated in the FcγR–IgG binding (Radaev et al., 2001), peptides with the sequences of the lower hinge regions of IgG could be potentially used as inhibitors of the receptors-IgG binding. This could be useful for the treatment of autoimmune diseases and pave a way for designing of new classes of therapeutic agents. To test this hypothesis, a series of binding competition experiments between the lower hinge peptides and Fc was carried out. Lower hinge peptides were immobilized on a sensor chip whereas a mixture of FcγRIII and variable concentrations of Fc was used as the analyte. The study shows that all lower hinge peptides compete with Fc for the binding to FcγRIII (Fig. 9). It remains to be seen if these peptides retain the same receptor blocking effect in vivo. It is likely that the sub-millimolar receptor binding affinities of these peptides are too low for them to be effective as therapeutic compounds. Further investigations to optimize the peptides are clearly needed in order to increase the potency of FcR inhibition. Nevertheless, our peptide binding study shows, in principle, peptides with the sequences of IgG lower hinge can be used as a prototype for designing of a new generation of therapeutic agents for treatment of some autoimmune diseases.

10. Role of Fc glycosylation in the recognition of antibodies by Fcγ receptors

The contribution of glycosylation of Fc to the function of immunoglobulins has been debated over the years (Jefferis et al., 1998; Kulczycki and Vallina, 1981; Lund et al., 1990; Pound et al., 1993). Early studies have demonstrated that, compared to the native IgG2a, deglycosylated IgG2a caused a mild reduction in the activation of complement component C1 and a drastic reduction in the activation of Fcγ receptors (Lund et al., 1990; Leatherbarrow et al., 1985). Later it was found (Pound et al., 1993) that deglycosylated IgG3 was capable of trigging human phagocyte respiratory burst at 80% of the level trigged by the glycosylated IgG3 despite a severe impairment in ADCC. However, unlike the impairment to the Fcγ receptor function, the removal of carbohydrates of IgE did not cause significant loss in FcεRI recognition (Kulczycki and Vallina, 1981; Basu et al., 1993). Structurally, the oligosaccharides attached to the conserved Asn 297 of IgG are a biantennary type with a core heptasaccharide, that consists of three *N*-acetylglucosamine (GlcNAc) and three mannose (Man), and variable fucose additions to the core (Jefferis et al., 1998). Unlike most glycosylations, these carbohydrates occupy a space between two chains of Fc and bury approximately 2400 Å^2 surface area of Fc. They are well ordered in all crystal structures of Fc with electron densities visible for most of the core sugar moieties (Deisenhofer, 1981; DeLano et al., 2000). In the FcγRIII–Fc and FcεRI–Fc complex structures, the carbohydrates on Fc contribute 100 and 50 $Å^2$, respectively, to direct interactions with the receptors (Radaev et al., 2001; Sondermann et al., 2000; Garman et al., 2000). To understand the apparent discrepancy between the known importance of this glycosylation to the function of FcγRIII and the absence of extensive direct contacts at the receptor-Fc interface, BIAcore binding studies were carried out using a deglycosylated IgG1 and its Fc fragment as the ligand (Radaev and Sun, 2001). Upon enzymatic deglycosylation, the FcγRIII affinity to IgG1 decreased 10–15-fold, while the receptor affinity to the Fc fragment was undetectable. The extensive surface area buried between the carbohydrate and Fc suggests a possible role for the carbohydrates as a surrogate hydrophobic core between the two chains of Fc thereby stabilizing the IgG lower hinge in an active receptor binding conformation. The removal of the carbohydrates may cause either a transition in the Fc conformation or an increased lower hinge flexibility. Consistent with the hypothesis, a structural change associated with aglycosylated IgG3 was previously observed by NMR spectroscopy in the vicinity of His 268 within the C_H2 domain (Lund et al., 1990). Alternatively, the carbohydrates, which contribute a small fraction of the receptor-Fc interface, increase the Fc binding affinity through direct contact (Burton, 1985).

11. A model for FcγRIII–IgG recognition and Fcγ receptor activation

On cell surface, FcR recognize intact immunoglobulins. The presence of the Fab portion of antibody is likely to impose restrictions to the receptor-Fc recognition. The only structure of an intact IgG available to date is a mouse antibody IgG2a (Harris et al., 1992). Since FcγRIII also recognizes mouse IgG2a, a model of this receptor-antibody complex was generated by superimposing the Fc part of the complex onto the Fc of the IgG2a (Fig. 10A). This receptor-antibody recognition model reveals that the receptor fits tightly and is nearly engulfed by the bound antibody.

The current structures of receptor-Fc complexes offer an insight to antibody-Fcγ receptor recognition, however the mechanism of receptor clustering and activation remains unknown. Two receptor clustering models can be proposed based on the current structural results, a simple avidity model and an ordered receptor aggregation model (Fig. 10B and C). The simple avidity receptor activation model assumes that the binding of oligomeric antigens by antibodies increases the avidity as well as the proximity of the receptors, which is sufficient for its activation. The ordered receptor aggregation model assumes that the binding of oligomeric antigens leads to the formation of an ordered receptor-ligand aggregation which further stabilizes the activation complexes. Recent imaging studies on T cells and NK cells receptor activation processes suggest that the formation of the so-called immune synapse is an ordered event (Monks et al., 1998; Davis et al., 1999). An ordered receptor-ligand aggregate was observed in the crystal lattice of a natural killer cell receptor in complex with its class I MHC ligand (Boyington et al., 2000). Such a receptor-ligand aggregate is not observed in the two forms of the current FcγRIII–Fc crystals. Instead, a parallel receptor aggregate was observed in the crystal lattice of FcγRIII in the absence

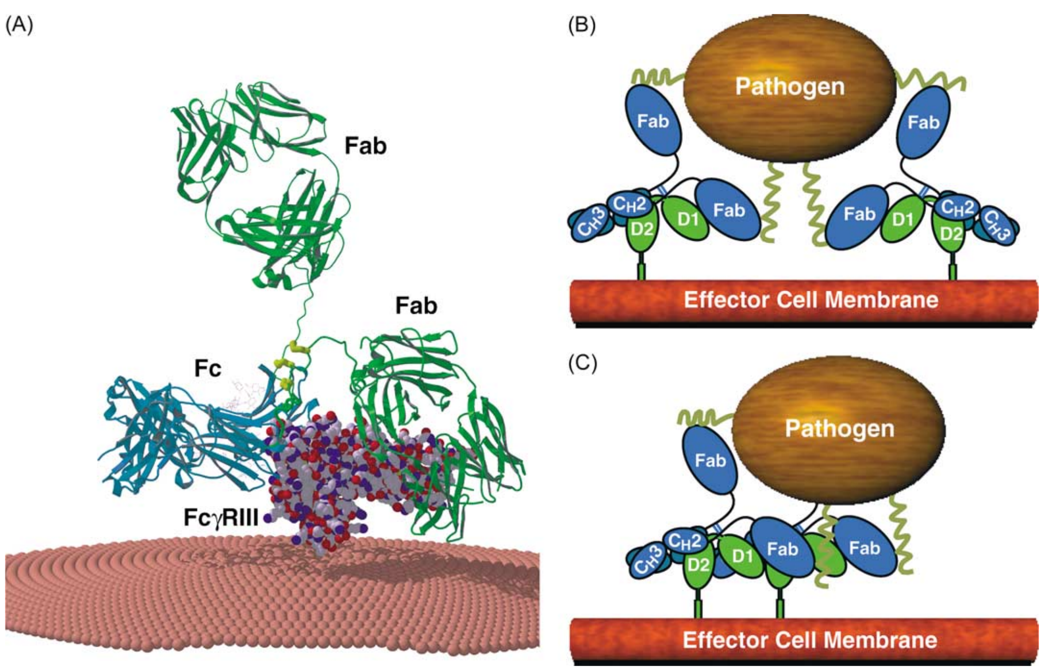

Fig. 10. Antibody-FcγRIII binding and ligand induced receptor aggregation model. (A) An intact antibody-FcγRIII binding model. The structure of the antibody is colored green (Fab) and cyan (Fc), FcγRIII is represented in CPK. The Protein Data Bank entry for the antibody coordinates is 1IGT. (B) A simple avidity model of antigen–antibody binding induced FcγRIII aggregation. (C) An ordered receptor aggregation model.

of Fc (Zhang et al., 2000). A superposition of the current complex structure onto this lattice receptor aggregate suggests that the clustering model would be compatible with the structure of the receptor-Fc complex (Fig. 10C).

In conclusion, it is worthwhile to emphasize that the 1:1 receptor-Fc binding stoichiometry highlights the importance of antigen in the receptor aggregation. In contrast to the high affinity FcγRI and FcεRI receptors, the binding of immunoglobulins to FcγRIII in the absence of antigen does not lead to receptor aggregation. It can be argued that a 1:1 receptor-ligand stoichiometry ensures the need for antigens and hence the immune complexes in promoting the receptor aggregation by eliminating the possibility of Fc mediated receptor aggregation as suggested in a 2:1 stoichiometry. Precluding receptor aggregation mediated by Fc alone also eliminates the potential deleterious effect of antibodies whose concentration in vivo are often much higher than that of antigen.

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